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Time-Course Effects of GA, GB, GD, GF and VX on Spinal Cord Cholinesterase and Acetylcholine Levels in Six Discrete Areas of the Rat Brain

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14. ABSTRACT
The time-course changes of spinal cord cholinesterase (ChE) and acetylcholine (ACh) levels in six discrete regions of the rat brain were studied and compared. Rats were injected subcutaneously with a 0.9 LD_{50} dose of GA, GB, GD, GF or VX. They were observed for symptoms of intoxication and head-focused microwave irradiated at 10 time points ranging from 5 minutes to 24 hours after compound administration. Brain tissue samples were homogenized and ACh analyzed using a high pressure liquid chromatographic (HPLC) method. Spinal cords were analyzed at each time point for degree of ChE inhibition. Results indicate that all five compounds produced observable signs of intoxication within 10 minutes after injection. Each compound inhibits ChE, resulting in increased levels of ACh within 10 minutes after administration, and the cortical and hippocampal areas were most sensitive to the effects of these compounds. Observable signs of intoxication, depression of spinal ChE activity and elevation of brain levels of ACh were similar for all of the compounds.

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INTRODUCTION

Inhibition of the enzyme acetylcholinesterase (AChE) with organophosphorus (OP) anticholinesterase agents results in an elevation of the neurotransmitter acetylcholine (ACh) at nerve terminal synapses (Brown et al., 1936; Eccles et al.,1942; Karczmar et al., 1970). This elevation of ACh at nerve terminals throughout the body is thought to be responsible for many of the observable manifestations of intoxication, such as muscle fasciculations, tremors, hypersalivation, convulsions and *status epilepticus* (Karczmar et al., 1970).

In this project, we used a high pressure liquid chromatograph (HPLC) method to analyze brain tissue homogenates to study the effect of five OP compounds, GA, GB, GD, GF and VX, on ACh levels in various brain regions. Although the toxicity of these five compounds is well characterized (Karczmar et al., 1970; Koelle, 1981; Maynard and Beswick, 1992), a comprehensive investigation using equitoxic doses to ascertain their effect on ACh levels in different brain regions and at different times has not been done. In addition, we looked at differences in the onset times and duration of rat spinal cord ChE inhibition in response to these five OP compounds.

The principal findings of this study showed that a 0.9 LD_{50} of any of the agents produced significant levels of central nervous system ChE inhibition within 10 min after injection. This inhibition was accompanied by increases in levels of ACh in all six brain regions studied. Observable symptoms produced by all the agents consisted of fasciculations, tremors, hypersalivation, convulsions and seizures. The cortex and hippocampus, respectively, were most sensitive to the effects of these five compounds. These two regions had the highest significant increases in ACh values. These findings are consistent with results obtained from a previous study with soman (Shih, 1982). In that study, the highest levels of ACh were also found in the cortex and hippocampus.

MATERIALS AND METHODS

Animal.

Male crl:CDBR Vaf/Plus Sprague-Dawley rats (*Rattus norvegicus*) supplied by Charles River Laboratories (Wilmington, MA) were used in this study. The average weight of the rats was approximately 250 g. They were housed individually in plastic cages (45 x 24 x 21 cm) in temperature (21 ± 2°C) and humidity (50 ± 10%) controlled animal quarters that were maintained on a 12-hr light-dark cycle (lights on at 6 AM). Laboratory rat chow and tap water were available *ad libitum*. At least 6 days were allowed for the animal to acclimatize to the environment of the animal room prior to experimental use.

Experimental Procedure.

This study was divided into ten experimental time points: 5,10, 20, 40 min and 1, 2, 4, 8,16 and 24 hrs. Each time point designated when the rats were euthanized after injection with one of the five test compounds or saline (control rats). Each of the five compounds and control animals were tested at each of these time points. For data analysis, we used a minimum of six rats (N=6) per group.

Rats were injected subcutaneously (sc) with a dose equivalent of 0.9 LD_{50} of GA (252 μg/kg), GB (112 μg/kg), GD (99 μg/kg), GF (189 μg/kg), VX (14 μg/kg) or saline as control (volume of 0.5 ml/kg). Following injection, rats were placed back into their cages and observed for signs of intoxication. At the predetermined time point, the rats were euthanized by a head-focused high intensity beam of microwave radiation (3.0 kW at 2.45 GHz for 1 second per 100 gram of animal) with a Gerling-Moore Metabostat System (Gerling-Moore, Inc., Santa Clara, CA). This procedure rapidly inactivates brain enzymes and creates an instant "snapshot" used to accurately determine concentrations of neurotransmitters in the brain tissues (Stavinoha et al., 1973). Following microwave irradiation, the brain was dissected into six different regions (brainstem, cortex, hippocampus, midbrain, cerebellum and striatum) following the procedures of Glowinski and Iversen (1966) for neurotransmitter assays. The spinal cord was also dissected, and the cervical segment, which had been affected by the microwave procedure, was discarded (Jimmerson et al., 1989). The thoraco-lumbar segment of the spinal cord was used for the ChE assay. Those animals that died before the designated time were not used for data collection.

Toxic Signs Scoring System.

Just prior to being euthanized, the level of intoxication was determined using a subjective scoring system based on visual observation of the subjects. The toxic signs scoring system (TSSS) consisted of six categories: motor, secretory/salivation, lacrimation, eye bulb protrusion, activity and coordination. The severity of intoxication within each category was based on a scale from 0-5 with 5 being the most severe intoxication. Each category used descriptive terms to help distinguish between the different levels of intoxication (Table 1). The maximum score any subject could attain would be 30 points (a 5 in each category). The overall point total for a group of rats (N=6) at a given time point would be 30 X 6=180. The point totals for rats in each group were averaged and standard errors calculated. The results were compiled into the histograms in Figure 8.

	0	1	2	3	4	5
MOTOR	normal	fasciculations		tremors		convulsion/ seizure
SALIVATION	normal	licking and chewing		wet around mouth		dripping saliva
LACRIMATION	normal	degree of clear tears			chromodacryorrhea	
EYE BULB PROTRUSION	normal	slight protrusion		moderate protrusion		severe protrusion
ACTIVITY	normal	hyperactive	hypo-responsive	unresponsive		prostrate
COORDINATION	normal	mild loss of coordination		moderate loss of coordination		loss of righting reflex

Table 1. Toxic Signs Scoring System (TSSS). Subjective scoring system designed to assess degree of intoxication through observation. Y-axis lists the six major categories for evaluation and 0-5 represent symptom severity.

Analysis of Brain Acetylcholine (ACh) Levels.

Following dissection, brain tissues were immediately weighed and homogenized in 1 ml of ice-cold 0.05 N perchloric acid. The homogenates were centrifuged for 20 min at 15,000 x g, and the supernatant was collected. To purify the supernatant for HPLC analysis, it was re-centrifuged through a microfilter of pore size 0.2 μm (Bioanalytical Systems, Inc., West Lafayette, IN).

Samples were analyzed with a BAS-200 HPLC with a reverse-phase C-18 column. Electrochemical detection was accomplished with a post-column immobilized enzyme reactor column that converts ACh into hydrogen peroxide, which is the detected entity (Bioanalytical Systems, Inc., West Lafayette, IN). Samples were quantified using external standards of concentrations 5.0 and 2.5 μmoles ACh. ACh levels were expressed as nmoles/g tissue.

Analysis of Spinal Cord Cholinesterase (ChE) Activity.

The spinal cord tissue was homogenized in 1% Triton X-100 in ice-cold saline (1:15; w:v) using 10 strokes with a glass/glass Potter-Elvehjem homogenizer. Homogenates were centrifuged at 4°C for 10 min at 15,000 x g, and the supernatant analyzed. Total ChE activity was determined by an automated method using a COBAS/FARA clinical chemistry analyzer (Roche Diagnostics Inc., Nutley, NJ), using acetylthiocholine as the substrate. The analytical procedure was based on the manual method of Ellman et al., 1961, and modified for the COBAS/FARA by Hobson et al., 1988.

Data Analysis.

The ACh and ChE values were calculated as nmoles/gram tissue and U/min/mg protein (U = 1 unit will hydrolyze 1.0 μmole of ACh to choline and acetate per minute at pH 8.0 at 37°C), respectively, and were expressed as percent of control values. Students' t-test was used initially to test for significant differences between ACh values for a given compound at each time point and those of control. Subsequently, ANOVA and the Tukey Test for multiple comparisons were used to compare time-course curves for control and all five compounds for each brain region. The hypothesis for each brain region, H1: sum (squared differences) for each compound and control are equal vs. H1a: sum (squared differences) are not equal. The sum of squared differences not significantly different from each other implies the curves are equal. Sigmaplot v. 2.0 and Sigmastat v. 2.0 for Windows 95 (Jandel Scientific, San Rafael, CA) were used for graphic representations and statistical analysis, respectively. Significance level was set at $p \leq 0.05$.

RESULTS

Toxic Signs and Lethality of GA, GB, GD, GF and VX.

All rats in this study received a subcutaneous 0.9 LD_{50} dose of GA (252 μg/kg), GB (112 μg/kg), GD (99 μg/kg), GF (189 μg/kg) or VX (14 μg/kg) based on the test model described earlier.

Figure 8 shows the observable physical symptoms of intoxication produced by each of the five OP compounds. Based upon the overall score for toxic signs (see Materials and Methods section, *Toxic Signs Scoring System*) GF appears to produce the largest values for intoxication, while GA has the lowest overall values for observable physical signs of intoxication. Regardless of the compound, the majority of rats began showing signs of intoxication by 10 min postinjection. The initial symptoms consisted of motor impairments ranging from fasciculations to convulsions and seizures. These motor impairments

were most often accompanied by hypersalivation, i.e., wet around mouth. Signs of intoxication beyond 2 hrs ranged from hypo-responsiveness to prostration.

The mortality figures for the 24-hr time point were GA = 0% (0/11); GB = 12% (1/8); GD = 22% (2/9); GF = 57% (8/14); and VX = 27% (3/11). The overall mortality for each compound without regard to individual time points was defined simply as the number of rats that died from compound intoxication divided by the total number of rats injected with the given compound: GA = 7% (5/75); GB = 5% (3/64); GD = 3% (2/63); GF = 48% (60/125); and VX = 4% (3/66).

For GA and GB, the first deaths occurred 20 min postinjection (2/8, and 1/7, respectively). The first deaths for GD and VX were recorded at 24 hrs (2/9 and 1/7, respectively). The first deaths occurred for GF at 60 min. (9/15).

Spinal Cord Cholinesterase (ChE) Activity.

Figure 1 illustrates the diminishing activity of spinal cord ChE after injection with any of the five compounds. The mean spinal cord ChE activity for the controls was 9.53 (± 0.22, SEM) U/min/mg protein. The ChE activity declined rapidly following injection with any of the compounds. At 5 min postinjection, ChE activity as a percent of control ± SEM percent of control was 66% ± 4% (VX), 68% ± 8% (GB), 72% ± 5% (GD), 73% ± 4% (GA) and 73% ± 9% (GF). At 10 min postinjection, ChE activity was 24% ± 3% (GF), 34% ± 7% (GD), 35% ± 8% (GB), 37% ± 4% (GA) and 41% ± 3% (VX). The low point of ChE activity for GD was at 10 min, while the low point was at 20 min for GA (12% ± 1%) and GB (22% ± 3%). The lowest ChE activity was at 40 min for VX (14% ± 1%) and 60 min for GF (22% ± 2%).

Time-Course Changes in Acetylcholine (ACh) Levels.

The control levels (mean ± SEM (N)) for ACh content (nmole/g tissue) in brain samples were striatum = 81.2 ± 2.8 (66), midbrain = 32.7 ± 1.0 (65), brainstem = 31.7 ± 1.0 (66), hippocampus = 30.0 ± 1.2 (65), cerebral cortex = 21.2 ± 0.9 (65) and cerebellum = 5.3 ± 0.3 (62).

Figures 2-7 illustrate the time-course effects of GA, GB, GD, GF and VX on ACh levels in various brain regions of the rat. Each figure shows 5 histograms, each representing a different compound. Data were converted to percentage of control for easy comparison.

In the cortex and hippocampus, the degree and duration of elevation of ACh were most apparent. In the cortex (Figure 3), at 10 min, all five compounds produced a significant ($P \leq 0.05$) increase in ACh levels (significance denoted by *). Between the 5-min and 8-hr time points, all the compounds showed some biphasic activity. ACh levels within the

hippocampus (Figure 4) also showed pronounced biphasic characteristics between 5 min and 8 hrs.

At 5 min postinjection, striatal levels of ACh for all compounds (Figure 7) decreased slightly. The histograms for GB, GD, GF and VX reveal a gradual increase in ACh levels out to 1 hr, while the histogram for GA shows sharp increases in ACh at 20 and 40 min and 2 and 4 hrs with a pronounced drop in ACh at 1 hr.

In the midbrain region (Figure 5), all of the ACh histograms are quite variable with no distinct pattern. There was an overall increase in ACh associated with all compounds even though ACh levels rose and fell across the range of time points.

ACh levels in the cerebellum (Figure 6) and brainstem (Figure 2) showed the weakest response to the five compounds. In response to GD, the overall ACh levels in the cerebellum decreased in comparison with controls. GF-induced increases in ACh levels in the cerebellum were unimpressive except for a large spike at the 4-hr time point (264% of control). GA also produced several spikes at the 20-min, 2- and 4-hr time points. Aside from these increases in ACh, the overall effect of the five compounds on ACh levels in the cerebellum was minimal. The response in the brainstem region was very similar to the cerebellum. The largest levels of ACh were produced by GA at the 20-min and 2-hr time points. VX also increased ACh to about 160% of control at 8 hrs. Aside from these increases, there is only a modest effect of the five compounds on ACh levels in the brainstem.

Comparisons of the overall changes in ACh (without respect to individual time points) generated by GA, GB, GD, GF and VX show significant differences ($P \leq 0.05$) between compounds in the various brain regions. In the cortex, all five compounds were significantly different from control. In addition, GA>VX, GB; GF>VX, GB, GD. In the hippocampus, all five compounds were significantly different from control and GF>VX. In the striatum, GA and GF were significantly different from control and GA>GB, GD, GF and VX. In the midbrain, GA, GF and VX were significantly different from control, while GA>GB and GD. In the cerebellum, there were no significant differences between any of the compounds and/or control. In the brainstem, GA>control, GB and GD.

A Comparison of Compound-Induced ACh Values to Control at Each Time Point.

Significant differences between compound-induced ACh values at each time point versus control were determined by Students' t-test. Significant values for ACh are labelled with an asterisk on Figures 2-7. The salient features of these data for each compound are described below.

GA: Cortical ACh values for time points 10, 20 and 40 min, 2 and 24 hrs were significantly greater than for controls. The 4-hr time point was not significant due to a large SEM. Hippocampal ACh values at 10, 20 and 40 min, 1 and 2 hrs were significantly different from controls. The ACh value at 4 hrs was not significant due to a large SEM. Significant striatal values for ACh were at 20 and 40 min, 1, 2 and 4 hrs. The midbrain had significant ACh values at 40 min, 2, 4 and 24 hrs. The cerebellum had significant values at 20 min, 2, 4 and 24 hrs. The brainstem had only 2 significant values for ACh at 40 min and 2 hrs.

GB: Cortical ACh values at 10 min, 20 min, 1, 2, 4 and 24 hrs postinjection were all significantly different from controls. Hippocampal ACh values were significantly different from controls at 10 and 20 min, 1, 4 and 16 hrs postinjection. The striatum had significantly different values from control at 1 and 2 hrs. The midbrain had significantly different values at 40 min and 1 hr. The cerebellum and brainstem each had only one value significantly different from control at 2 hrs and 4 hrs, respectively.

GD: Cortical ACh values were significantly different from controls at 10 min, 1, 4 and 24 hrs. Hippocampal values were significantly different from controls at 2, 4 and 16 hrs postinjection. The striatum had four values significantly different from controls at 5 min,10 min, 40 min and 2 hrs. The midbrain and cerebellum each had one significant value at 40 min and 24 hrs, respectively. The brainstem had no values for ACh that were significantly different from controls.

GF: Cortical ACh values were significantly different from controls from 10 min to 8 hrs. The 24-hr time point was also significantly different from controls. The hippocampus had five significantly different ACh values at 10 min, 1, 2, 4 and 8 hrs. The striatum had significantly different values from controls at 40 min and 1 hr. The midbrain had three significantly different values at 10 min, 1 and 8 hrs. The cerebellum had no significant values. The brainstem had ACh values significantly larger than control values at 10 min and 24 hrs postinjection.

VX: Cortical ACh values from 10 min to 1 hr and 4 and 24 hrs were significantly different from controls. Hippocampal values at 10 min, 40 min, 1 and 4 hrs were significantly different from controls. Striatal ACh values at 5 min and 1 hr were significantly different from controls. Midbrain values were significantly different from controls at 10 min and 1 hr. Both cerebellum and brainstem regions had one significant ACh value at 60 min.

A Comparison of Maximal ACh Values vs. Brain Region For GA, GB, GD, GF and VX.

The comparisons are shown in Table 2. For each compound, Table 2 lists the statistically significant maximum ACh value (ACh_{max}) and the time it

occurred in each of the six brain regions. These ACh_{max} values were rank ordered from high to low among brain regions for each compound. For all compounds, cortical ACh values were the largest, ranging from 198% (VX) to 429% (GF) above controls. The hippocampal region was rank ordered second for GB, GD and GF with values ranging from 197% (GB) to 242% (GF) above controls. The striatum (246%) and midbrain (148%) were rank ordered second for GA and VX, respectively. The hippocampus was rank ordered third for both GA and VX.

Rank Order of ACh_{max}

GA	Cortex 351% 20 min	Striatum 246% 2 hr	Hippo 209% 20 min	Brainstem 177% 2 hr	Midbrain 166% 2 hr	Cerebell 162% 20 min
GB	Cortex 290% 4 hr	Hippo 197% 4 hr	Striatum 153% 2 hr	Midbrain 128% 1 hr		
GD	Cortex 410% 4 hr	Hippo 230% 4 hr	Striatum 129% 2 hr			
GF	Cortex 429% 4 hr	Hippo 242% 4 hr	Midbrain 161% 1 hr	Striatum 142% 1 hr	Brainstem 125% 10 min	
VX	Cortex 198% 10 min	Midbrain 148% 10 min	Hippo 143% 10 m, 1 hr	Striatum 128% 1 hr	Brainstem 125% 1 hr	Cerebell 123% 1 hr
GD (1982)* Shih, T.-M. (1982).	Cortex 421% 2 hr	Hippo 196% 16 hr	Cerebell 154% 40 min	Striatum 138% 2 hr	Brainstem 134% 20 min	Midbrain 132% 1 hr

Table 2. Comparison of significant maximal ACh values (ACh_{max}) versus brain region for GA, GB, GD, GF, VX and GD (1982)*. This Table illustrates for each of the compounds, from left to right (1), the rank order of brain regions from largest to smallest significant ACh_{max} values; (2) the significant ACh_{max} values calculated as a percentage of control; (3) the time at which the ACh_{max} occurred in that region following intoxication. An empty box indicates no significant ACh_{max} values.

DISCUSSION

In the present study, the five OP compounds were administered in equitoxic doses equivalent to 0.9 LD_{50}. The 24-hr mortality for GA was 0% (0/11); for GB, 12% (1/8); for GD, 22% (2/9); for GF, 57% (8/14); and for VX, 27% (3/11). Subsequent reanalysis of GA revealed a 25% decrease in the stock concentration. Due to the change in stock concentration and lack of observable signs of intoxication, the 20-min, 40-min, 2-hr, 4-hr and 24-hr groups for GA were repeated. Reanalysis of the other four compounds showed no changes in stock concentrations; therefore, deviations from the expected 45% mortality could be due to several other factors: seasonal variation in animal response, weight range of the animals and/or the use of historical LD_{50} values.

Injection of a 0.9 LD_{50} dose of any of the five compounds initiated a series of events consisting of a decrease in total spinal cord ChE activity (Figure 1) coupled with an increase in brain regional ACh (Figures 2-7). Jimmerson et al. (1985,1989) found good correlation ($R=0.94$-0.98) between spinal cord ChE activity and ChE activity in the six major brain regions studied. Thus, in addition to an increase in brain regional ACh, extrapolation of results from previous studies allows us to predict a decrease in ChE activity in these same regions. The graphic ChE profiles for the five compounds were very similar in that they all show a rapid inhibition of ChE activity within 10 min of injection ranging from 59% for VX to 76% for GF. This rapid inhibition of ChE activity is consistent with the results of a study by Maxwell et al. (1987) in which the time-course of ChE inhibition in anesthetised rats injected with 0.84 LD_{50} of soman (GD) was studied. In that study, soman produced a rapid maximal inhibition of ChE activity (<20% of control) within 15 minutes of intramuscular (im) soman administration. In another study using VX, Maxwell et al. (1996) found that the polarity of VX under physiological conditions makes it likely that CNS related signs of intoxication would occur later than similar symptoms produced by the other less polar compounds. Examination of the rates of inhibition of spinal cord ChE in Figure 1 does not show any indication that VX had a slower entry into the CNS. At 10 min postinjection it appears that all five compounds produced very similar levels of inhibition. Figure 1 shows the differences in ChE activity levels at times beyond 10 min. These variations in ChE activity levels are probably due to the incomplete ChE inhibition caused by the 0.9 LD_{50} dose of the compounds. This dose would also be expected to affect the corresponding ACh values. In fact, Figures 2-7 reveal a recurring pattern of ACh spiking. This pattern occurs in all six brain regions and with all five OP compounds tested. This peak and valley pattern might be due to the incomplete inhibition of ChE resulting in oscillating levels of ACh.

All of the agents produced observable signs of intoxication within 10 min after injection. This timeframe corresponds to the extensive ChE inhibition at

10 min postinjection. GF produced the most rapid onset and longest sustained duration of severe observable toxic signs, while GA and VX had a slower, less intense onset and duration (Figure 8).

The symptoms of intoxication were similar for all five compounds, although the severity of the symptoms varied between animals. Initial symptoms ranged from muscle fasciculations to tremors to convulsions and usually included some degree of hypersalivation. At times beyond 2 hrs, intoxication progressed to a state of severe motor impairment with symptoms ranging from hypo-responsiveness to prostration to a severe loss of the righting reflex.

The cortex and hippocampus have been identified by the data contained in Table 2 and Figures 2-7 as the brain regions most sensitive to the effects of the five compounds. All five compounds produced the highest ACh_{max} values in the cortex (Table 2) with values ranging from 198-429% above control. After the cortex, the next highest significant percent of control values for ACh_{max} were produced in the hippocampus (GB, GD, GF), striatum (GA) or the midbrain (VX).

In any discussion of the brain regional increases in ACh values, we must distinguish between the increases in terms of percent of control changes in ACh values and changes in basal ACh values in terms of nmoles/g of tissue. Table 2 shows the brain regions with the largest percent of control increases referred to as ACh_{max}. If we look at the ACh control data in terms of nmoles/g of tissue (See Results section, Time-Course Changes in Acetylcholine (ACh) Levels), the brain regions with the largest percent of control changes in ACh, namely, the cortex and hippocampus, are areas with lower basal levels of ACh in terms of nmoles/g of tissue.

It is up to the investigator to decide which of the changes is more relevant to assessing the effects of these compounds on the various brain regions. One issue relevant to this question is whether high levels of ACh per gram of tissue or the percent of change in control ACh levels may have any relationship to the subsequent levels of neuropathology. Neuropathology data has been collected on the effects of soman (Petras, 1981; McLeod et al., 1984) and other seizure-producing chemicals such as bicuculline (Blennow et al., 1978) on various brain regions of the rat. These experiments have shown that areas such as the cortex (21.2 $\pm$ 0.9 nmole/g tissue), hippocampus (30.0 $\pm$ 1.2) and midbrain (thalamus and subthalamic structures; 32.7 $\pm$ 1.0) incur substantial damage, while regions such as the cerebellum (5.3 $\pm$ 0.3), brainstem (31.7 $\pm$ 1.0), and striatum (81.2 $\pm$ 2.8) incur little or no neuronal damage. It appears that neither absolute levels of ACh nor percent of control values for any isolated brain region would be a useful diagnostic tool for predicting the occurrence of neuropathology. As postulated by McDonough and Shih (1997), elevated excitatory amino acid levels at later stages of the seizure, rather than preliminary elevation of ACh, may play a role in the production of neuropathology.

CONCLUSIONS

All five compounds produced observable signs of intoxication by 10 min postinjection. Initial symptoms of intoxication were similar for all the compounds and consisted of muscle fasciculations, tremors and hypersalivation. Beyond 2 hrs, intoxication progressed to a state of severe motor impairment. ChE profiles for the five compounds (Figure 1) reveal a rapid inhibition of spinal cord ChE levels >50% within 10 min postinjection. Inhibition of ChE was associated with a corresponding increase in brain regional ACh. The areas most sensitive to the effects of these five compounds were the cortex and the hippocampus, but large increases in brain regional ACh are probably not a reliable indicator of impending neuropathology.

Figure Legends

Figure 1: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on spinal cord (thoraco-lumbar segment) cholinesterase activity. Values expressed as percent of controls. Each time point represents N = 6.

Figure 2: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on brainstem ACh values in rats. Values expressed as a percentage of controls. Each time point represents N = 6. Error bars represent SEM's. An asterisk (*) represents a significant value ($P \leq 0.05$) compared with controls.

Figure 3: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on cortical ACh values in rats. Values expressed as a percentage of controls. Each time point represents N = 6. Error bars represent SEM's. An asterisk (*) represents a significant value ($P \leq 0.05$) compared with controls.

Figure 4: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on hippocampal ACh values in rats. Values expressed as a percentage of controls. Each time point represents N = 6. Error bars represent SEM's. An asterisk (*) represents a significant value ($P \leq 0.05$) compared with controls.

Figure 5: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on midbrain ACh values in rats. Values expressed as a percentage of control. Each time point represents N = 6. Error bars represent SEM's. An asterisk (*) represents a significant value ($P \leq 0.05$) compared with controls.

Figure 6: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on cerebellum ACh values in rats. Values expressed as a percentage of controls. Each time point represents N = 6. Error bars represent SEM's. An asterisk (*) represents a significant value ($P \leq 0.05$) compared with controls.

Figure 7: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on striatum ACh values in rats. Values expressed as a percentage of controls. Each time point represents N = 6. Error bars represent SEM's. An asterisk (*) represents a significant value ($P \leq 0.05$) compared with controls.

Figure 8: Time-course effect of a 0.9 LD_{50} dose of GA, GB, GD, GF and VX on observable signs of intoxication in rats. Values represent average point totals for members of each time point grouping (N = 6). Error bars represent SEM's. Points for each rat were determined using the Toxic Signs Scoring System.

Spinal Cord ChE Activity

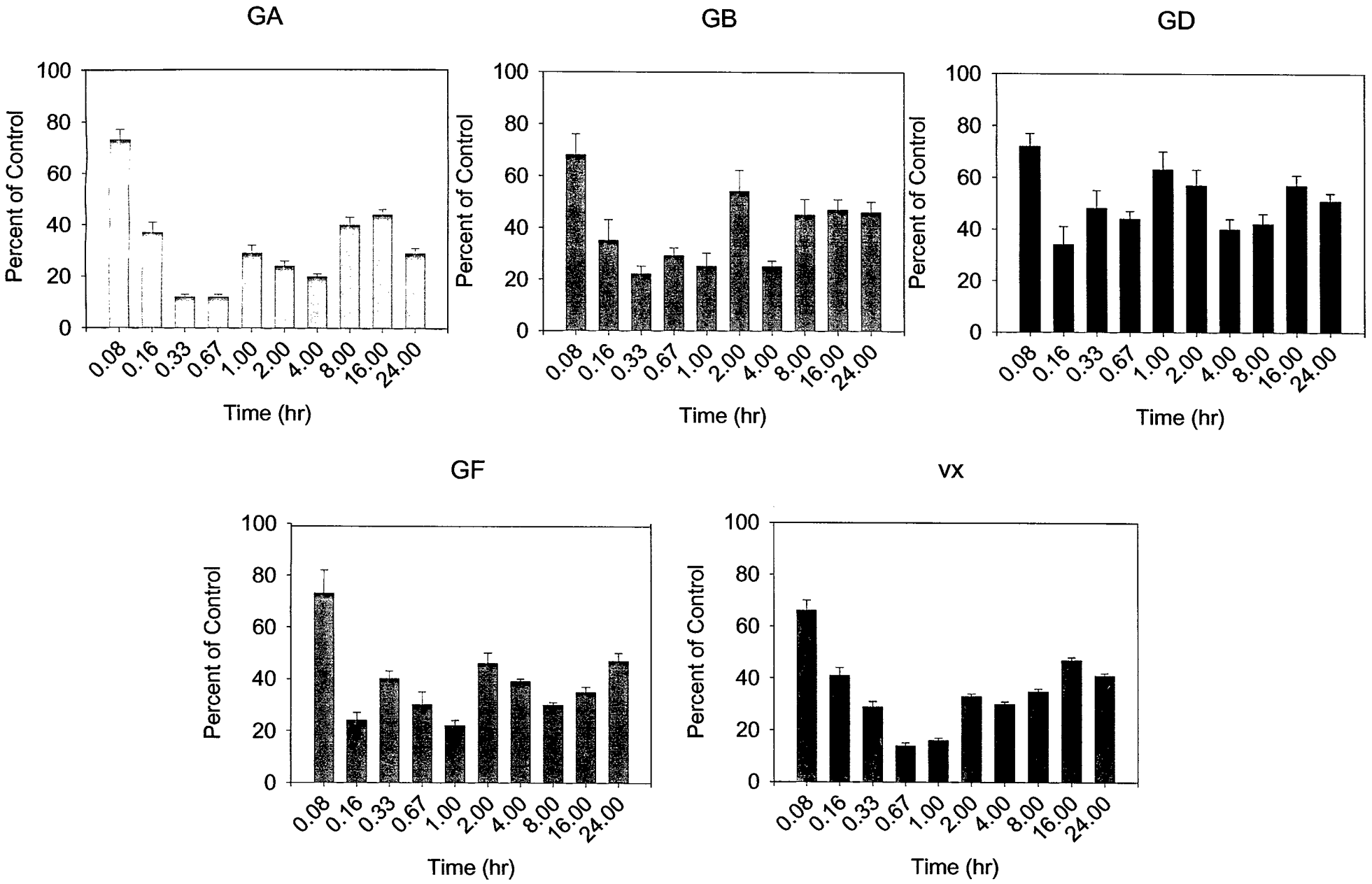

Figure 1

Brainstem ACh

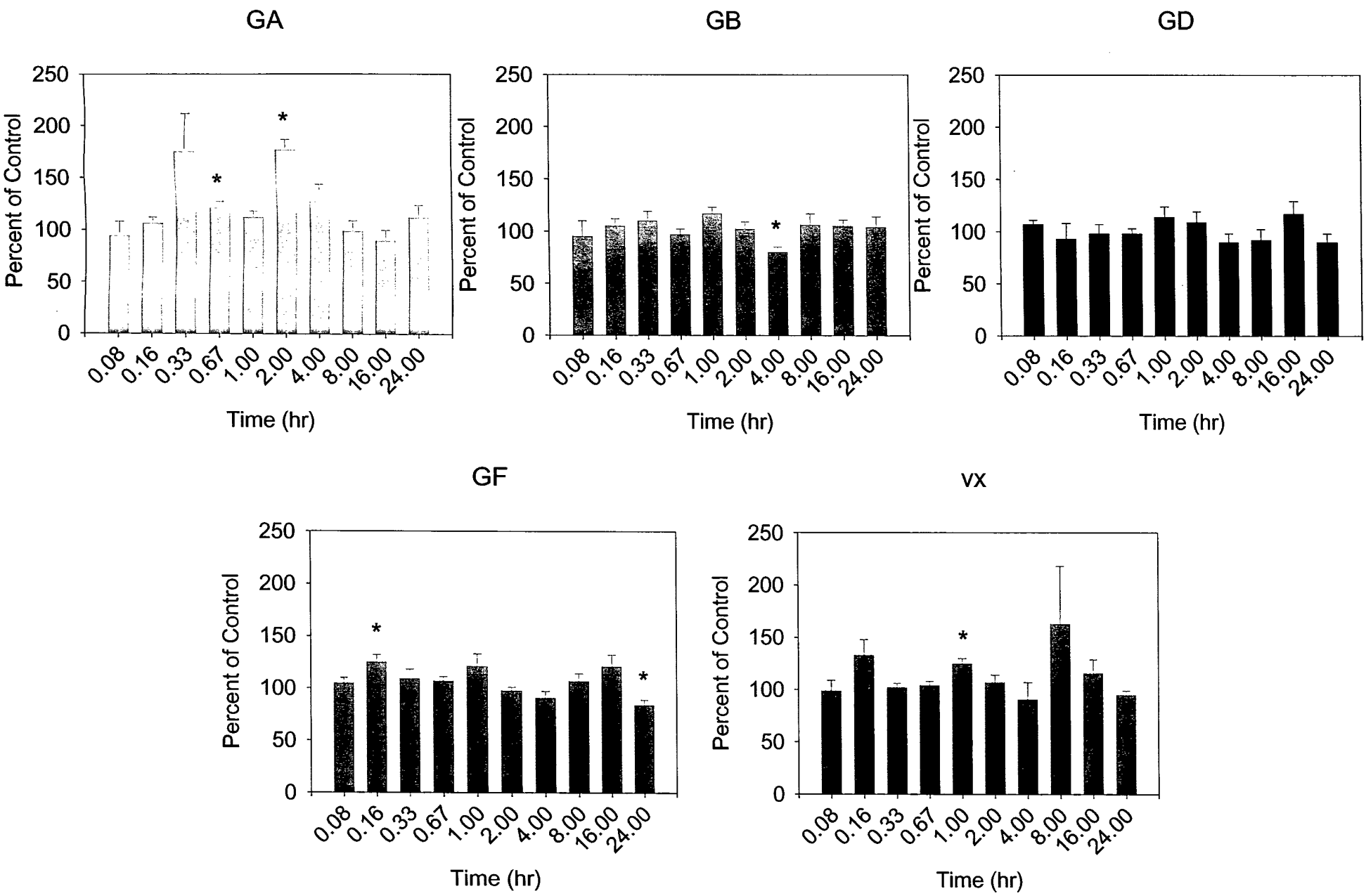

Figure 2

Cortex ACh

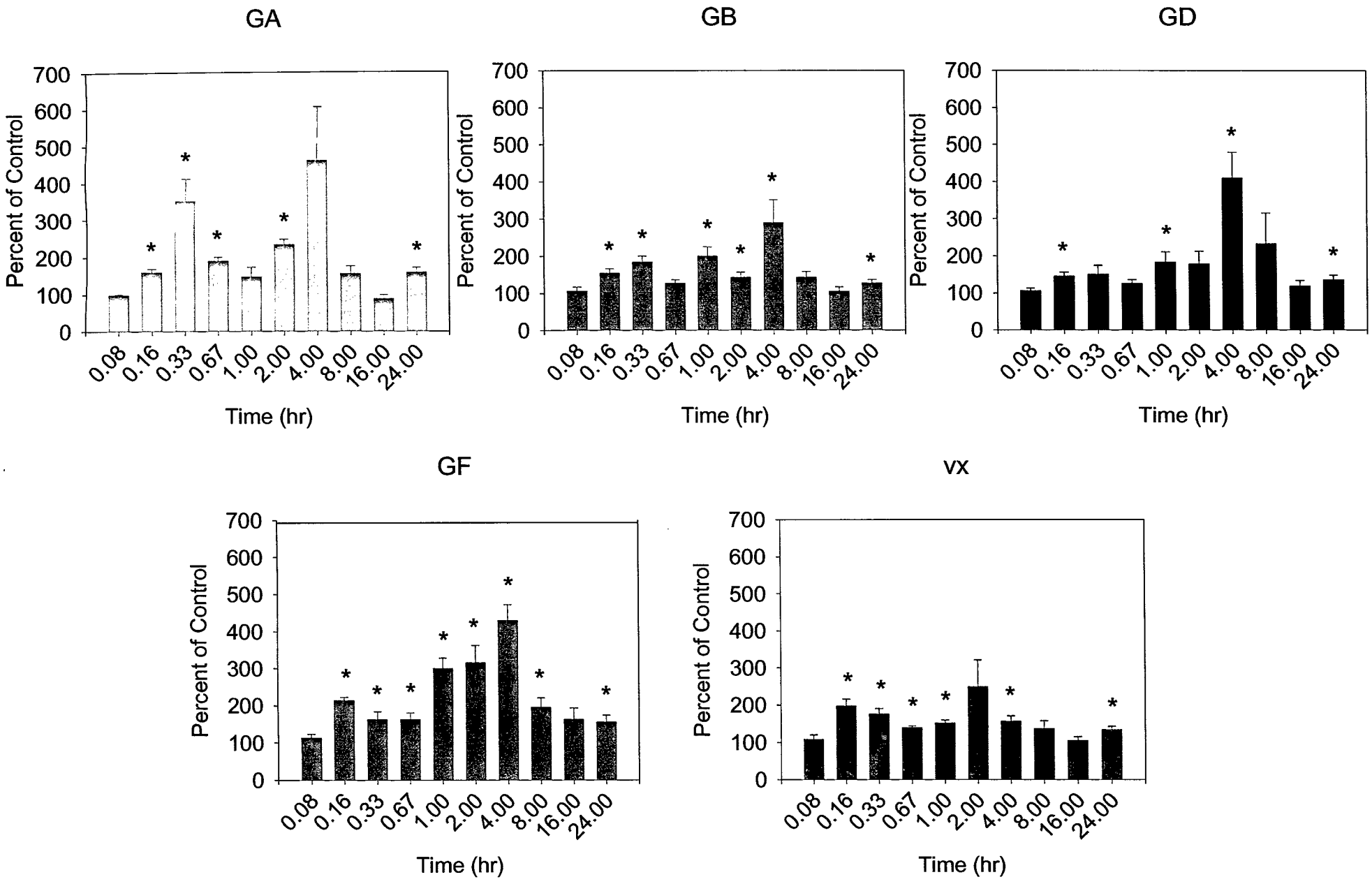

Figure 3

Hippocampus ACh

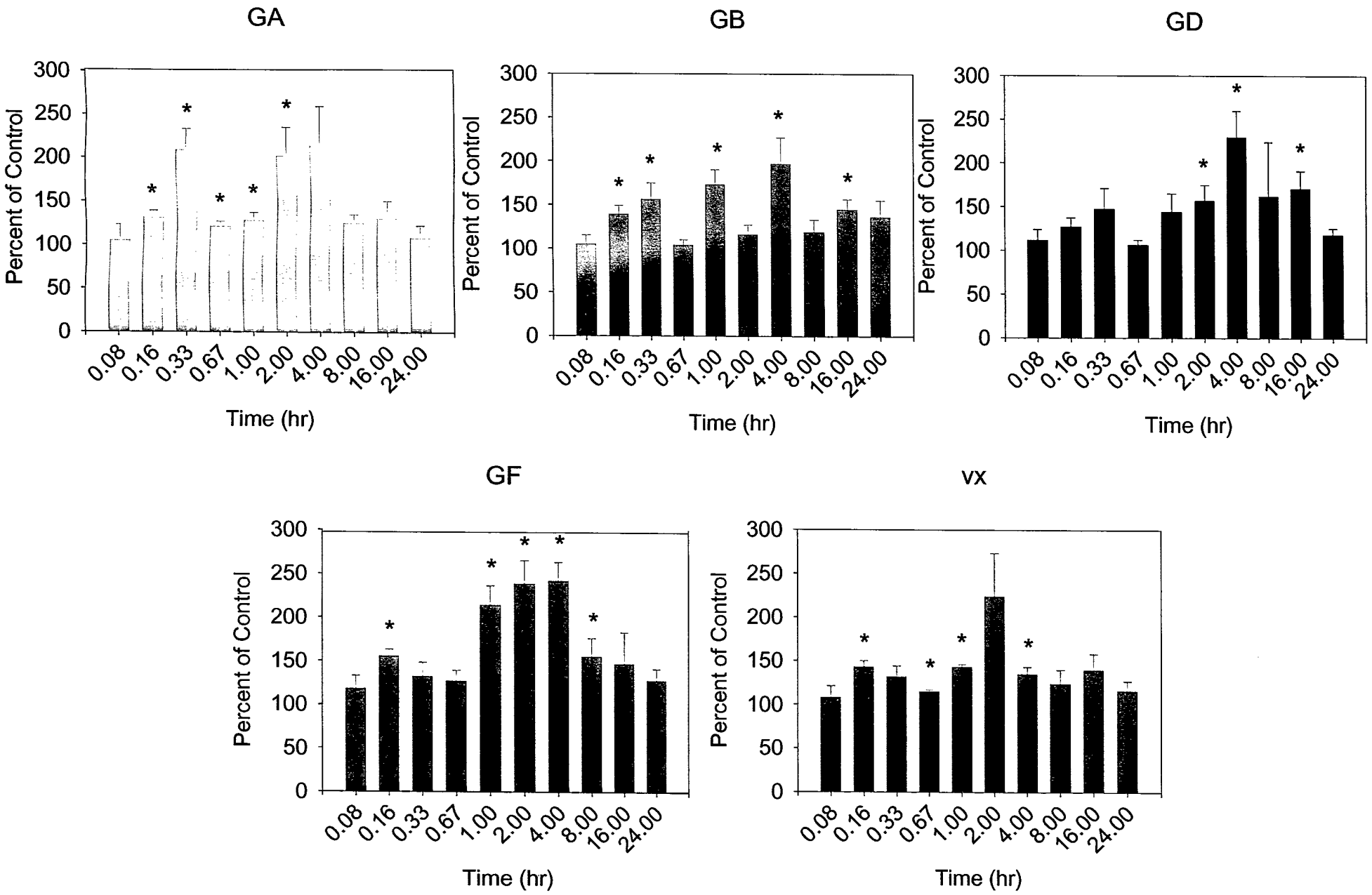

Figure 4

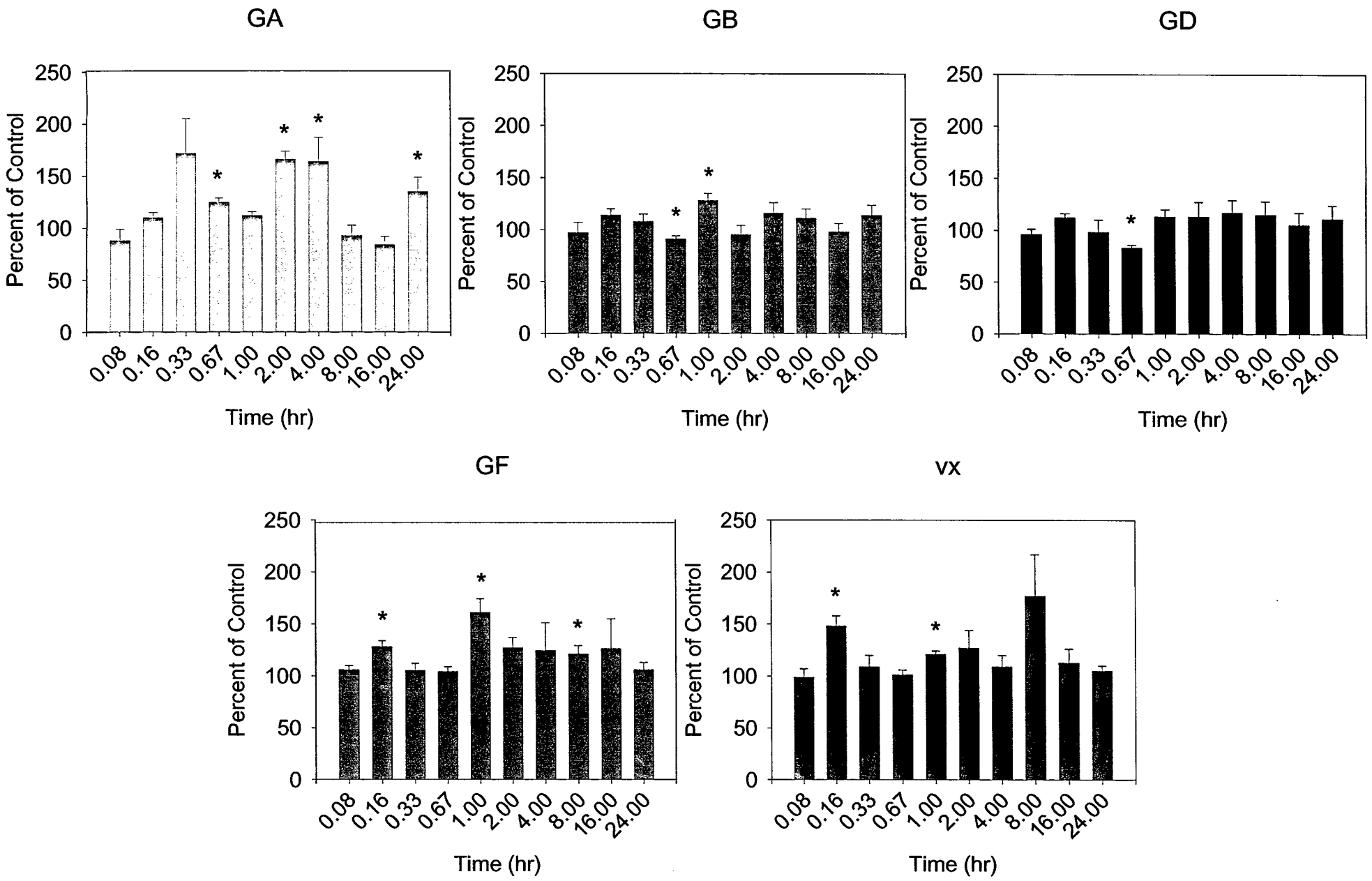
Midbrain ACh
GA
GB
GD
GF
VX
Percent of Control
Time (hr)
0
50
100
150
200
250
0.08
0.16
0.33
0.67
1.00
2.00
4.00
8.00
16.00
24.00

Figure 5

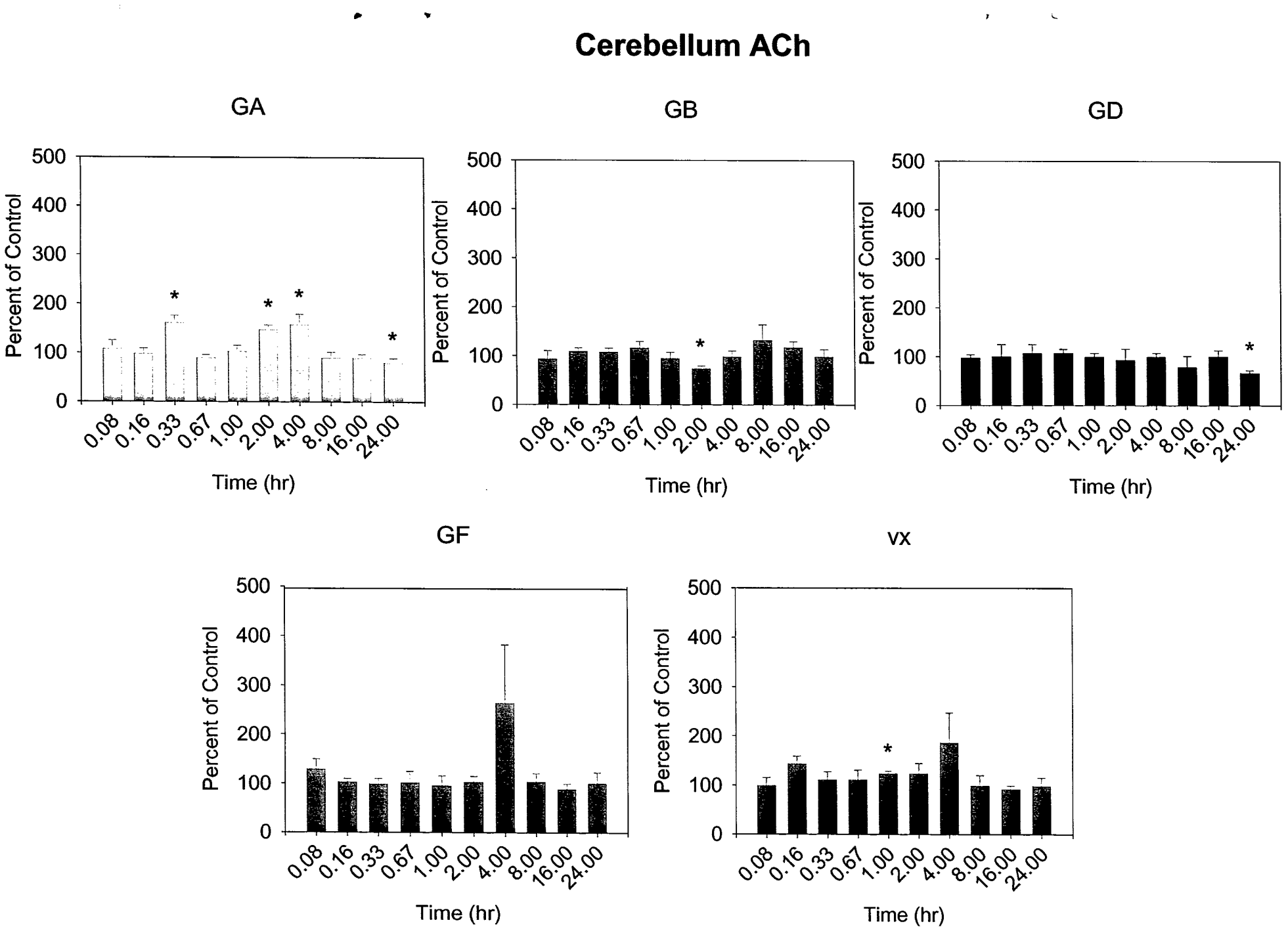
Cerebellum ACh
GA
GB
GD
GF
VX
Percent of Control
Time (hr)
0
100
200
300
400
500
0.08
0.16
0.33
0.67
1.00
2.00
4.00
8.00
16.00
24.00
*

Figure 6

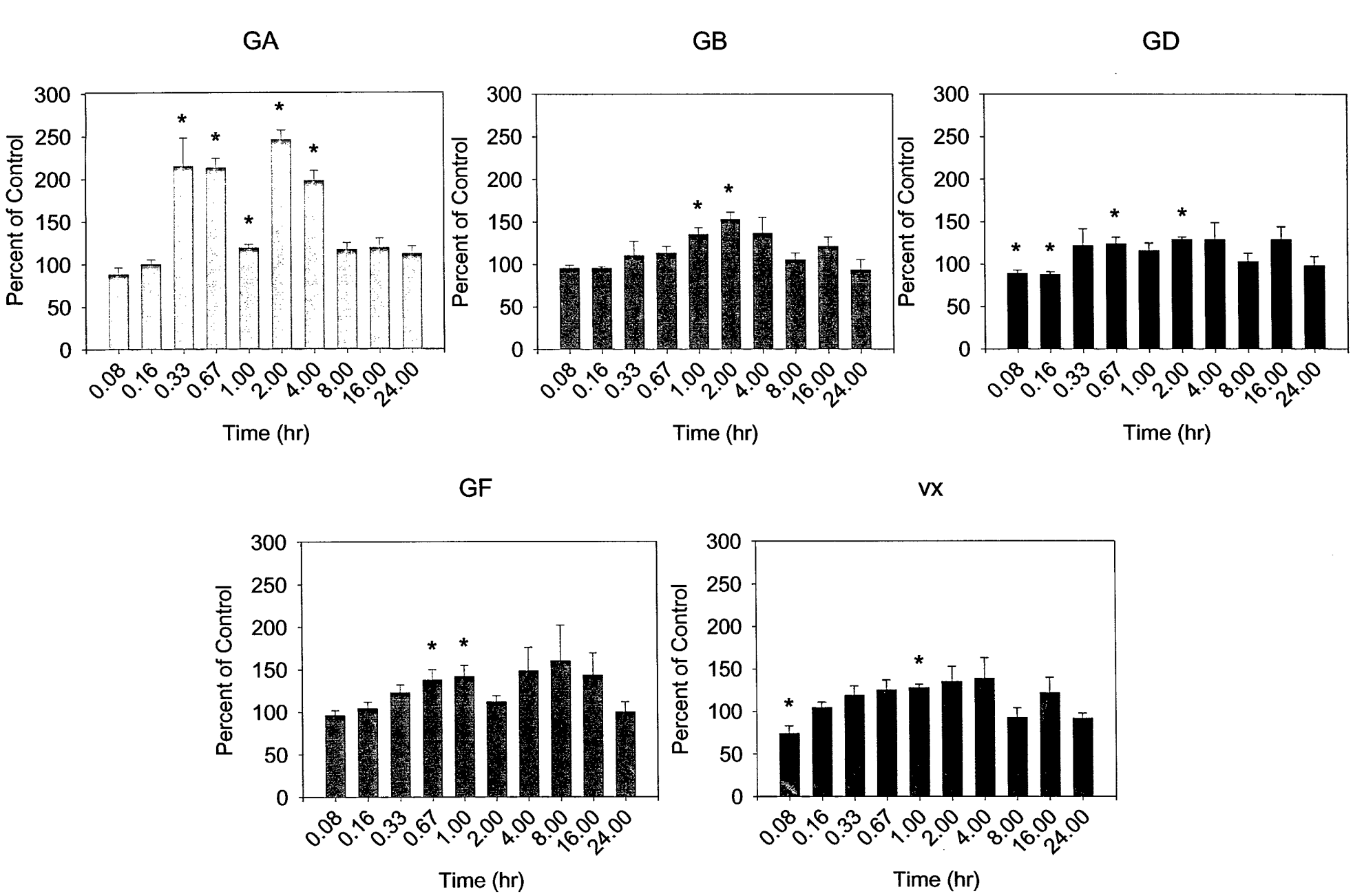
Striatum ACh
GA
GB
GD
GF
VX
Percent of Control
Time (hr)
0
50
100
150
200
250
300
0.08
0.16
0.33
0.67
1.00
2.00
4.00
8.00
16.00
24.00

Figure 7

Observable Toxic Signs

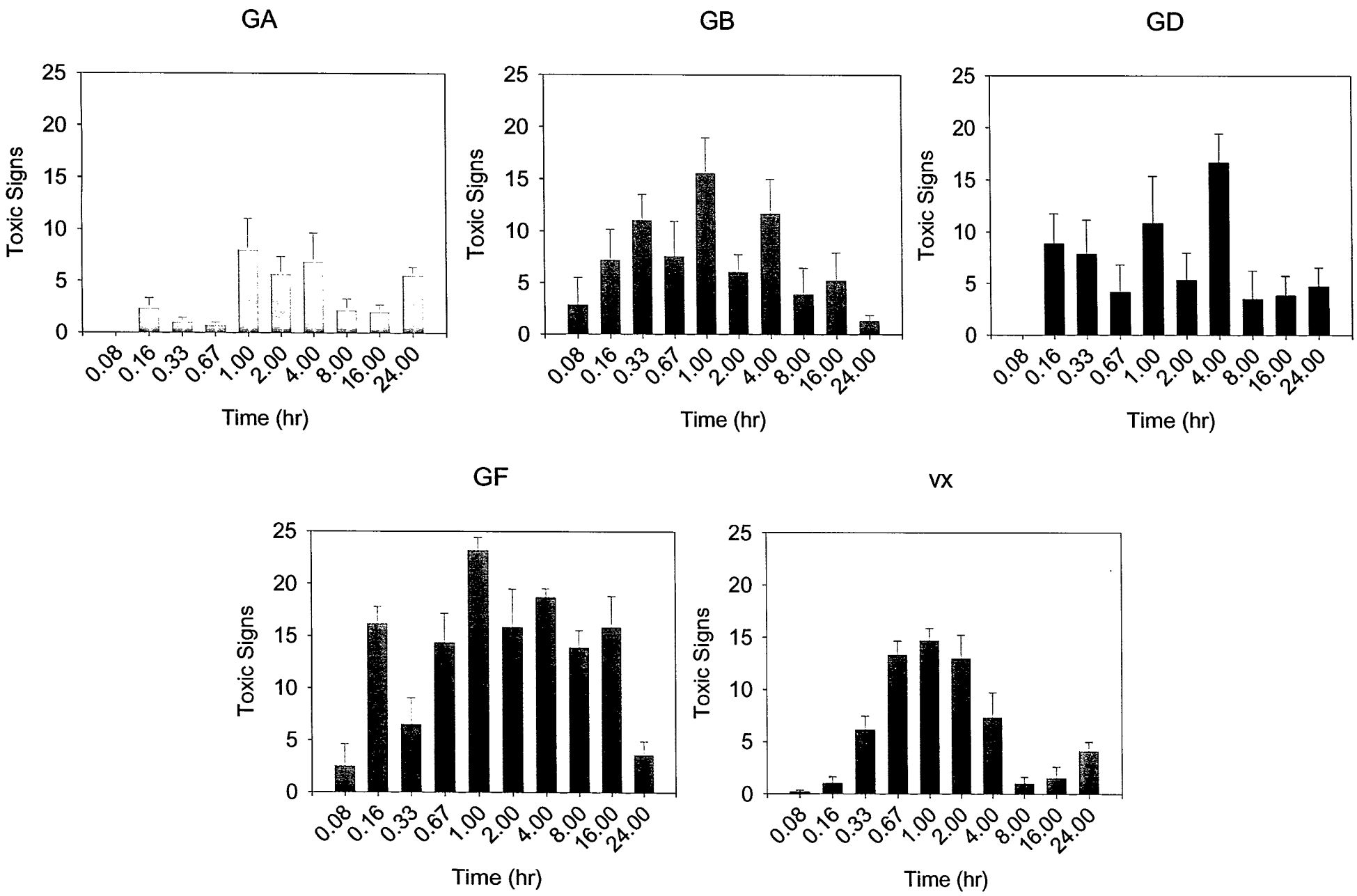

Figure 8

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